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EDITORIAL

Contrast-enhanced ultrasound as noninvasive diagnostic images that anyone can easily understand

Kazushi Numata[1]

Beautiful images may be universal. Everyone can feel something and be moved when he or she sees beautiful images even though they cannot communicate using language. Even if images are not beautiful, easy-to-understand ones are trustworthy for everyone. Generally, the golden standard for noninvasive diagnostic images is pathological findings. For nearly 10 years, we have been evaluating pathological diagnoses once a month using biopsy specimens under the guidance of a doctor with more than 30 years of experience who specializes in liver pathology. Therefore, regarding easy-to-understand images for diagnoses, I describe the relationship between the pathological findings and the noninvasive diagnostic images such as conventional ultrasound (US) and contrast-enhanced ultrasound (CEUS) images of hepatic lesions, as an example.

Hepatocarcinogenesis is a complex and multistep process. A spectrum of focal hepatic lesions develop within the liver parenchyma, ranging from low-grade and high-grade dysplastic nodules (DN) to pathologically early hepatocellular carcinoma (HCC) and pathologically advanced HCC. According to the International Working Party criteria [1] and International Consensus Group for Hepatocellular Neoplasia (ICGHN) [2], HCCs were classified histologically into early HCC (Fig. 1), well-differentiated HCC, moderately differentiated HCC, or poorly differentiated HCC (Fig. 2). The features examined were the degree of cellular atypia, structural atypia, pattern of the reticular fibers, degree of neovascularization, degree of stromal invasion, and the ductular reaction [3]. The cell density, the nuclear size and morphology, the nuclear-cytoplasmic ratio, and alterations of the trabecular structure were evaluated in sections stained with hematoxylin and eosin (HE). Decrease in the reticular fibers was evaluated using silver staining. The ICGHN arrived at the following consensus regarding the histological diagnostic criteria for early HCC [2]: it stated that the presence of tumor cell invasion into the intratumoral stroma (portal tract) [4] should be recognized as the most important histopathologic finding for the diagnosis of early HCC. The diagnosis of intratumoral stromal invasion requires Victoria Blue staining [5]. The ductular reaction, which refers to the staining of bile ducts in the periportal tract resulting from the change of bile metabolism in cases of HCC, was evaluated by immunostaining for cytokeratin (CK) 7 [6]. Neovascularization seen in HCC lesions was examined by immunostaining for CD34, which is one of the commonly used endothelial cell markers [7].

Since pathological diagnosis is performed based on various staining methods other than the above, a certain amount of specimen is required. With a resected specimen, a sufficient amount of specimen can be obtained. However, sometimes small hepatic lesions are not resected; therefore, US-guided tumor biopsies are often used. In contrast, collecting a specimen from a hepatic lesion using biopsy is not easy. It is necessary to puncture the target lesion accurately, and even if the lesion can be punctured, it may not always be possible to obtain a sufficient amount of specimen depending on the tissue. Even if a sufficient amount is obtained, an early HCC cannot be diagnosed by HE staining alone, as described above. It is necessary to prepare many slides with immunohistochemical markers. Even with such preparations, proper pathological diagnosis cannot be achieved without sufficient knowledge and experiences to evaluate them. Therefore, there are some variations in the pathological diagnosis. However, in general, many doctors make decisions based on the results of the pathological reports, without looking at the pathological findings, i.e., without sharing the process of pathological diagnosis. In such a situation, a discrepancy between the diagnosis based on pathological findings and that based on the noninvasive diagnostic images frequently occurs. Therefore, it is important to

✉ Kazushi Numata
kz-numa@urahp.yokohama-cu.ac.jp

[1] Gastroenterological Center, Yokohama City University Medical Center, 4-57 Urafune-cho, Minami-ku, Yokohama, Kanagawa 232-0024, Japan

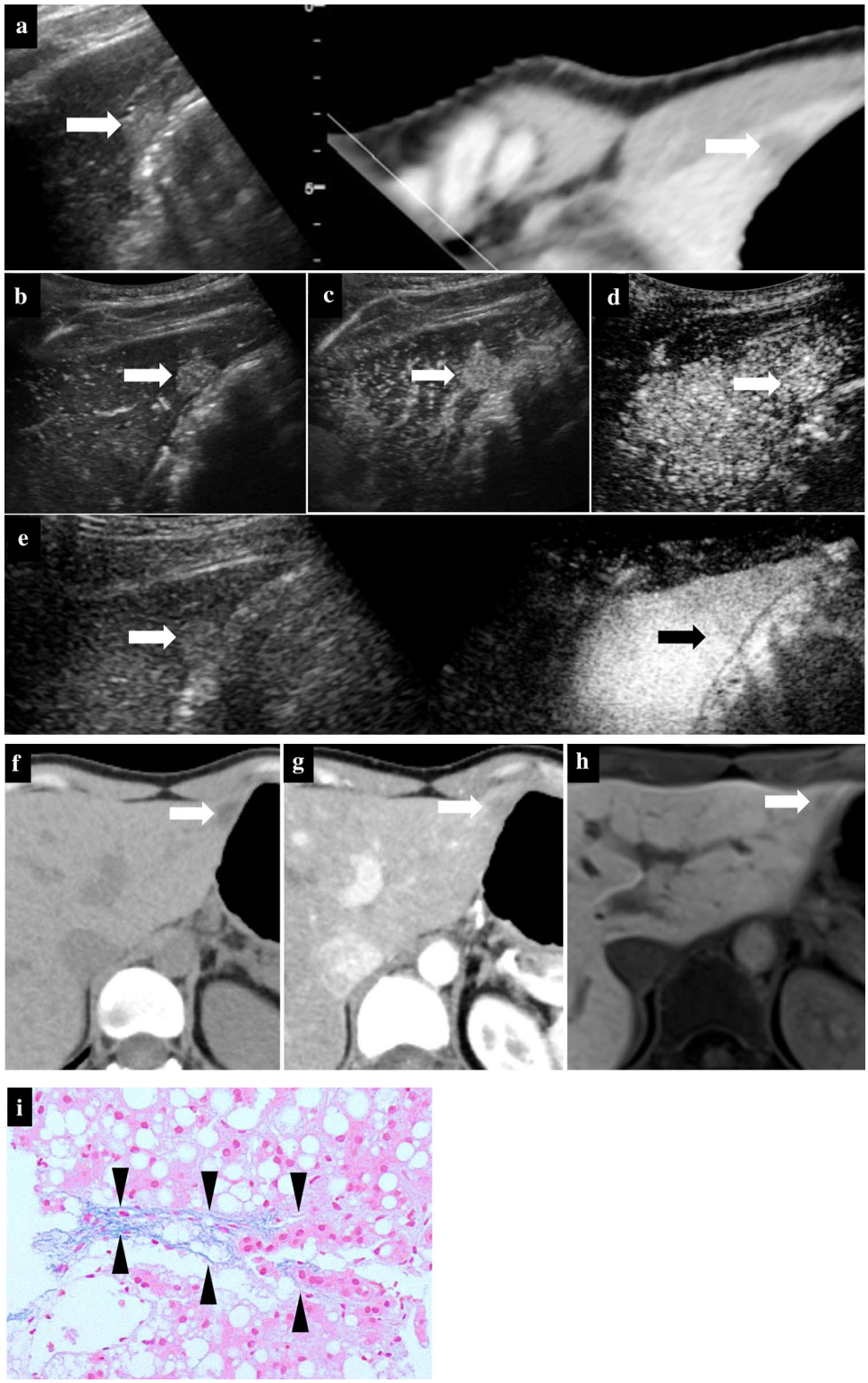

Fig. 1 A case of hypervascular early HCC (maximum diameter, 14 mm) in segment II of the liver. **a** Fusion imaging combining conventional US (left side) and arterial phase contrast-enhanced CT (right side) on a single screen. Conventional US shows a hyperechoic nodule. Neither the halo nor the mosaic sign is positive. **b**–**d** Arterial phase Sonazoid CEUS images obtained using low MI harmonic imaging shows hypervascularity with centripetal vessels (**b**, **c**), and the lesion is seen as an isovascular lesion in the subsequent portal phase image (**d**). **e** Post-vascular phase CEUS image obtained with high MI contrast imaging showed an isoechoic tumor (right side). The left side is conventional US as a reference. **f**–**g** The lesion is seen as a low-density area in segment II on non-enhanced CT (**f**), and as a non-high-density area on arterial phase contrast-enhanced CT (**g**). The hepatobiliary phase of EOB-MRI shows a slightly hypointense area in segment II (**h**). An arrow indicates the HCC lesion (**a**–**h**). Hematoxylin–eosin staining shows slight cellular atypia, a mild increase in the nuclear-cytoplasmic ratio, and hypercellularity with fat components (data not shown). **i** Victoria blue staining of the tumor area shows elastic fibers surrounding the portal tract in blue (arrowheads). Positive stromal (portal tract) invasion, namely, cancer cells within the portal tract, is compatible with the diagnosis of early HCC

obtain easy-to-understand noninvasive diagnostic images for hepatic lesions that can be diagnosed only with it.

Regarding CEUS, the main imaging technique is low mechanical index (MI) contrast imaging to evaluate tumor vessels and tumor enhancement of hepatic lesions. The reference conventional US of low MI contrast imaging is fundamental B-mode US. The spatial resolution of the fundamental mode is insufficient to confirm the location of the target lesion itself. Additionally, frame rates of 10–15 frames per second are also insufficient to evaluate tumor

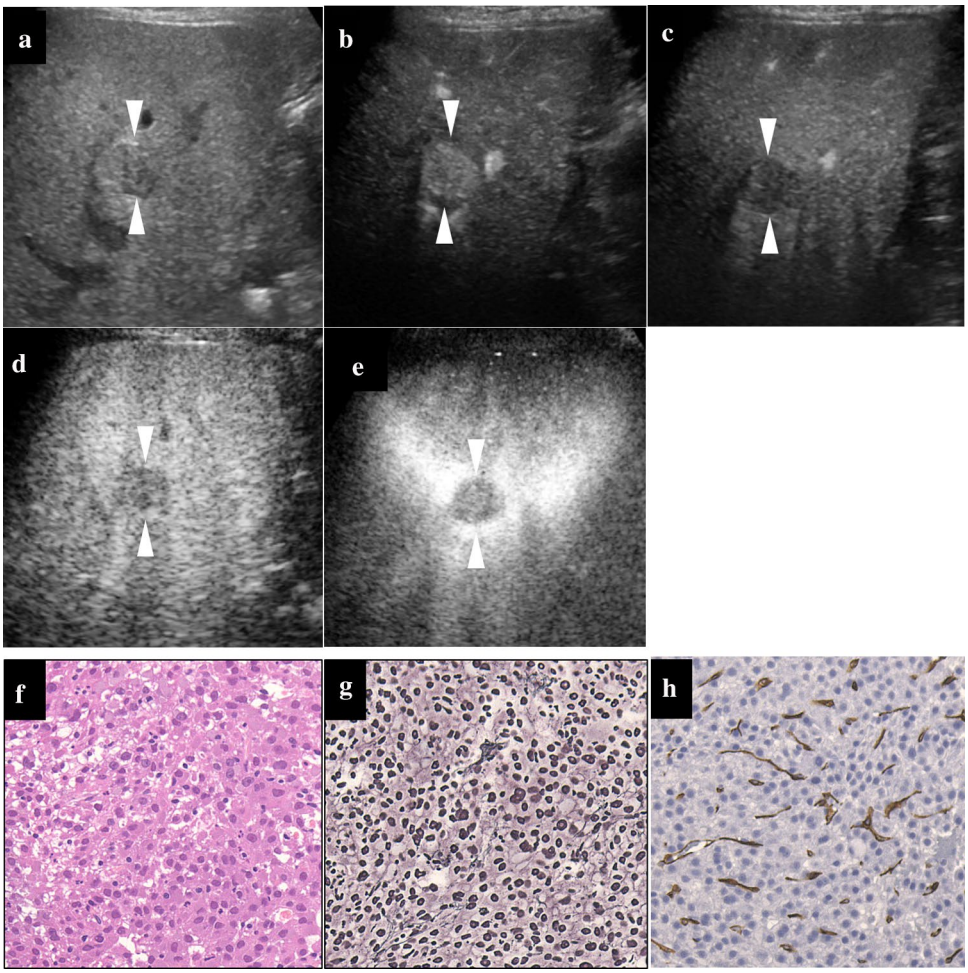

Fig. 2 A case of hypervascular poorly differentiated HCC (maximum diameter, 20 mm) in segment V of the liver. Conventional US shows a slightly hypoechoic nodule with a halo (**a**). **b**, **c** Arterial phase Sonazoid CEUS images obtained using low MI harmonic imaging show hypervascularity (**b**), and the lesion is seen as a hypovascular lesion in the subsequent portal phase image (**c**). **d** Post-vascular phase CEUS image obtained with low MI contrast imaging shows a hypoechoic tumor. **e** After the imaging mode is switched to high MI contrast imaging, the post-vascular phase also shows a hypoechoic tumor. The arrowheads seen in **a**–**e** indicate the margin of the lesion. **f** Hematoxylin–eosin staining. The tumor cells are arranged in solid sheets, and the trabecular structure is lost. The nuclei are enlarged and have bizarre shapes. **g** Silver staining of a tumor area. The reticular fibers are almost completely lost. **h** Positive immunoreactivity of the sinusoidal capillaries, indicative of diffuse CD34 expression. This lesion was histopathologically diagnosed as a poorly differentiated HCC

vessels because tumor enhancement is too fast to evaluate tumor vessels.

Recent conventional gray-scale US, represented by wideband phase inversion harmonic gray-scale imaging, provides a high spatial resolution and deep penetration with high frame rates of 24–30 frames per second. In this US imaging modality, once the target lesion is detected, the MI can be manually reduced to around 0.18–0.28. Subsequent Sonazoid (Daiichi Sankyo, Tokyo, Japan) injection offers detailed evaluation of the tumor vessels and tumor staining in real time due to the high frame rate [8–10]. This imaging technique is called "low MI harmonic imaging," which is one of the alternative techniques of Sonazoid CEUS imaging [11]. Using this imaging technique during the portal and post-vascular phases, we have to take care that Sonazoid microbubbles are easily destroyed compared with low MI contrast imaging due to the higher frame rates. The mainstay of evaluation of tumor vessels using both low MI contrast imaging and low MI harmonic imaging will be to take a video, rewind it frame by frame, and evaluate the frames as still images [9].

Focal nodular hyperplasia (FNH) can be diagnosed simply by detecting a spoke wheel pattern, namely, centrifugal vessels during arterial phase CEUS (Figs. 3, 4). In representative cases of early HCC, the lesion shows no halo on conventional US and isoechogenicity during post-vascular phase CEUS [3]. In addition, in the majority cases of poorly differentiated HCC, the CEUS images show hypervascularity with centripetal vessels during the arterial phase, and the lesion is seen as a hypovascular lesion in the subsequent portal and post-vascular phases [3]. These findings of CEUS images can, to some extent, presume pathological diagnosis. In contrast, in cases of hypervascular early HCC and hypervascular well-differentiated HCC having the same CEUS findings such as hypervascularity during the arterial phase and subsequent isovascularity and isoechogenicity during the portal and the post-vascular phases and no halo on conventional US, it is needed to carry out a histopathological examination because of difficulty of differentiation by conventional US and CEUS images alone. Therefore, in this specific case, CEUS as noninvasive diagnostic images may play a supplementary role in the pathological diagnosis,

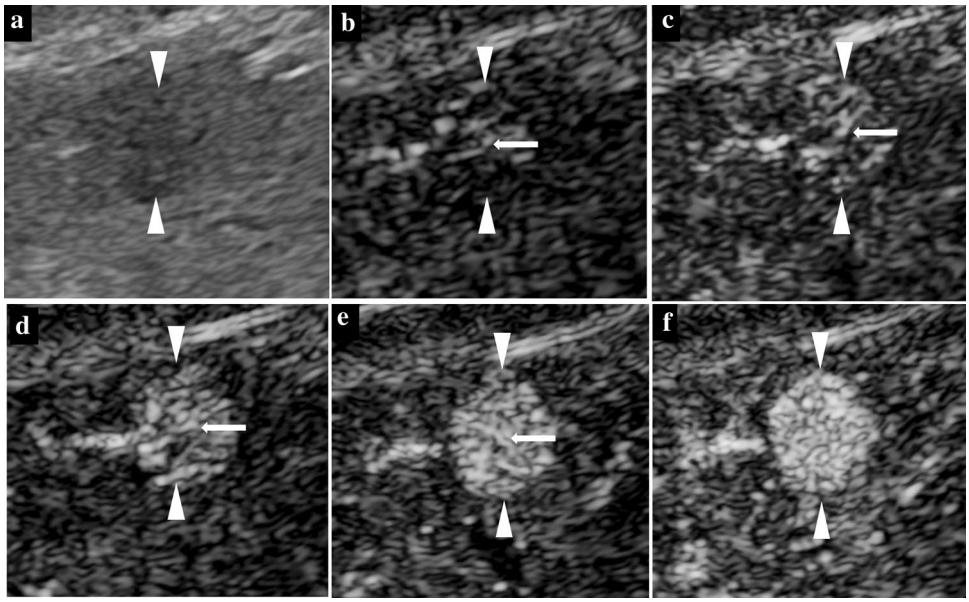

Fig. 3 Sonazoid CEUS images obtained using low MI contrast imaging in a case of focal nodular hyperplasia (maximum diameter, 12 mm) in segment III of the liver. **a** The fundamental mode as conventional US and as a reference of CEUS image shows a slightly hypoechoic lesion with an ill-defined margin. **b–f** Arterial phase Sonazoid CEUS images using an amplitude modulation as low MI contrast imaging show homogeneous hypervascular enhancement with centrifugal vessels. An arrowhead indicates a faint spoke wheel pattern, namely, the tumor vessels running from the center to the periphery (centrifugal vessel pattern). The arrowheads seen in **a–f** indicate the margin of the lesion

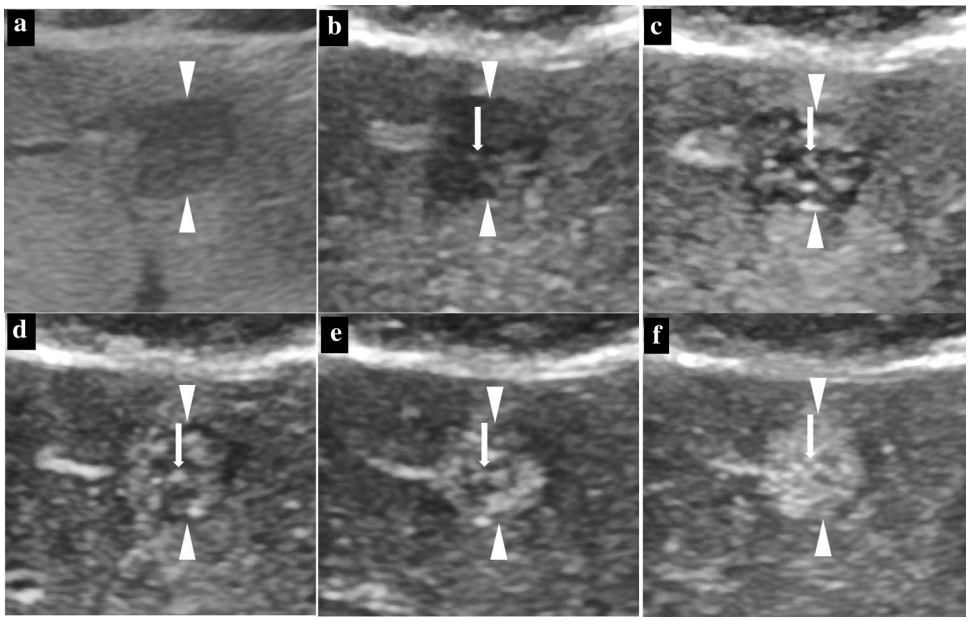

Fig. 4 Sonazoid CEUS images obtained using low MI harmonic imaging in the same case seen in Fig. 3. **a** Native tissue harmonic imaging as conventional US shows a hypoechoic lesion with a well-defined margin. Compared with conventional US seen in Fig. 3, the margin of the lesion is clearer, which results in confirmation of the accurate location of the lesion. After confirming the location of the lesion, we decrease the mechanical index of this imaging manually. After Sonazoid injection, CEUS findings such as tumor vessels and tumor enhancement were evaluated while confirming the location of the lesion. **b–f** Arterial phase Sonazoid CEUS images using low MI harmonic imaging show homogeneous hypervascular enhancement with centrifugal vessels. Compared to the tumor vessels seen in Fig. 3, a clearer spoke wheel pattern is seen (arrowhead). The arrowheads seen in **a–f** indicate the margin of the lesion

no matter how excellent the easy-to-understand images are. However, we try to obtain easy-to-understand images of tumor vessels and tumor enhancement that can play a complementary role in the pathological diagnosis. These efforts will help to reduce the frequency of biopsies from hepatic lesions.

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